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Examiner	:	Regina M. DeBerry
Applicant	:	Gregory M. Fahy, Ph.D.
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For	:	GROWTH HORMONE THERAPY AND RELATED METHODS AND PHARMACEUTICAL COMPOSITIONS

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

DECLARATION OF GREGORY M. FAHY

I, Gregory M. Fahy do hereby declare the following:

Example of Thymic Regeneration in a Normal Human Subject

Thymic regeneration protocol. Beginning on February 13th, 1996 (day 0), I (at the time a 46.8-year-old male) began self-administration of 0.018 mg/kg of hGH (Humatrope, Eli Lilly & Company, Indianapolis, Indiana) by subcutaneous injection near midnight four days per week (Tuesday, Thursday, Saturday, and Sunday). I also ingested 200 mg/day of pharmaceutical grade dehydroepiandrosterone (DHEA; College Pharmacy, Colorado Springs, Colorado) at about the same time as I received each hGH injection. This regimen was continued through March 19th. Pre- and post-treatment blood samples were collected on Wednesdays [February 7th (baseline), February 21st (day 8), and March 20th (day 36)] to evaluate the response of pertinent metabolites to the treatment regimen.

Thymic imaging and lymphoid volume estimation. Prior to treatment (on February 8th, 1996), mediastinal magnetic resonance (MR) imaging was performed (Shady Grove M.R.I. Associates, P.A., Rockville, Maryland) at a field strength of 1.5 Tesla, with 6 mm spacings between adjacent image planes. The MR imaging series was repeated on March 13th, 1996, after a total treatment period of 29 days, to provide an evaluation of potential thymic regeneration. The two largest thymic cross sections at each sampling time were carefully matched to ensure near-identical loci before and after treatment and scanned at a resolution of

1200 dpi with background illumination. The resulting digital image files were optimized for display of gray scale information and arranged for direct side-by-side visual comparison using Adobe Photoshop Elements 2.0 and annotated.

The thymus was most clearly demonstrable in three contiguous image planes both before and after treatment. Total thymic volume and total volume of thymic lymphoid mass were therefore roughly estimated as follows. First, image distance scales were calibrated against measurements of the human subject's thorax. Second, the mean image anterior-to-posterior dimensions and widths of each of the three clearest post-treatment thymic cross sections were estimated. Finally, the average of the three mean widths and the average of the three mean anterior-posterior dimensions were multiplied together and the product was multiplied by 1.8 cm (the thickness of the three combined sections) to estimate total thymic volume. Pre-treatment thymic volume was calculated from post-treatment volume times the ratio of pre-treatment to post-treatment thymic mean image surface area as determined from image analysis. Absolute total thymic and thymic gray zone cross-sectional areas were determined using the free Scion Image image analysis program (version beta 4.0.2, available at http://www.scioncorp.com).

Results. Figure 1 presents a side-by-side comparison of the thymus at two different locations before (A,B) and after (C,D) treatment. All images showed the classical anatomical position of the thymic remnant between the aorta and the sternum as known in the art. All images showed a combination of visually white (high intensity signal) mass indicative of adipose tissue substitution for lymphoid tissue, a typical observation for this age range, and visually gray (intermediate intensity signal) mass, representing lymphoid or functioning thymic mass. However, the second set of images distinctly and consistently shows more total thymic cross-sectional area and more gray thymic cross-sectional area, and a definite darkening of some gray regions following treatment can be appreciated in Figure 1. These results are given in quantitative form in Table 1. In addition, similar changes were seen in the third major

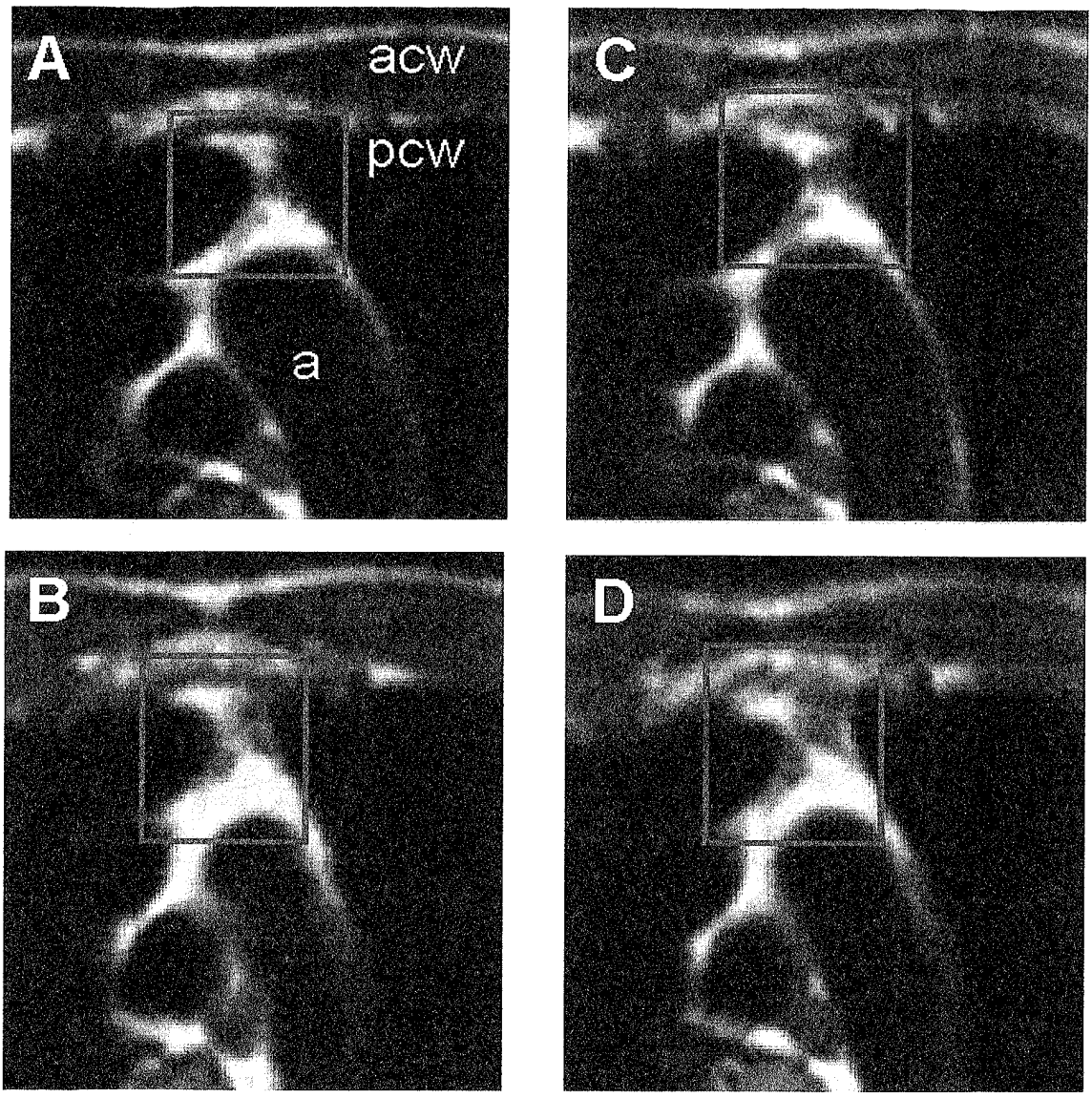

Figure 1. MRI scans of thymus (within the blue boxes) before (A,B) and after (C,D) approximately one month of HGH + DHEA therapy. acw = anterior chest wall; pcw = posterior chest wall; a = aorta.

adjoining thymic cross section as well as in a smaller contiguous potential thymic cross section (data not shown).

Table 1: Image Analysis Results for Figure 1[a]

Image Characteristic	Image A (pre)		Image C (post)	Image B (pre)		Image D (post)
Thymus area (cm^2)	5.5		7.0	5.1		6.0
% change		26.5			16.9	
Total Gray area (%)	38.9		64.1	51.5	66.9	
% change		64.7			30.0	
Dark gray area (%)	17.8		27.4	8.1		26.6
% change		53.5			228	

[a]Obtained using enlarged images.

The total thymus volume was estimated at about 10.4 ml after treatment, and at about 8.5 ml before treatment. Assuming dark gray cross section represents 100% lymphoid tissue and light gray cross section represents 50% lymphoid tissue, the total thymic lymphoid volume can be estimated from these data as being about 2.5 ml before treatment and 4.8 ml after treatment. Assuming a density of 1 g/ml, the former value is within the normal range for a subject of this age (mean plus 97.8% confidence limit ~3.1g; see Judd and Bueso-Ramos, Combined true thymic hyperplasia and lymphoid hyperplasia in Graves' disease. Pediatric Pathol. 1990;10:829-836, copy enclosed). However, the post-treatment value is well in excess of the normal range (well over three standard deviations above the mean; see Figure 2, which is a plot derived from Judd and Bueso-Ramos' data) and therefore meets the standard proposed by Judd and Bueso-Ramos for establishing thymic hyperplasia in a random individual. The percent increase in total thymic lymphoid (functional) mass induced by the Fahy art was 92%.

As indicated in Table 2, IGF-1 levels rose to the upper limit of normal throughout the documented times of the experiment. Other blood tests indicated increased triglyceride levels indicative of fat mobilization by IGF-1, reduced total and low density cholesterol levels with no change or an increase in HDL-cholesterol levels, and unaltered thyroid hormone and

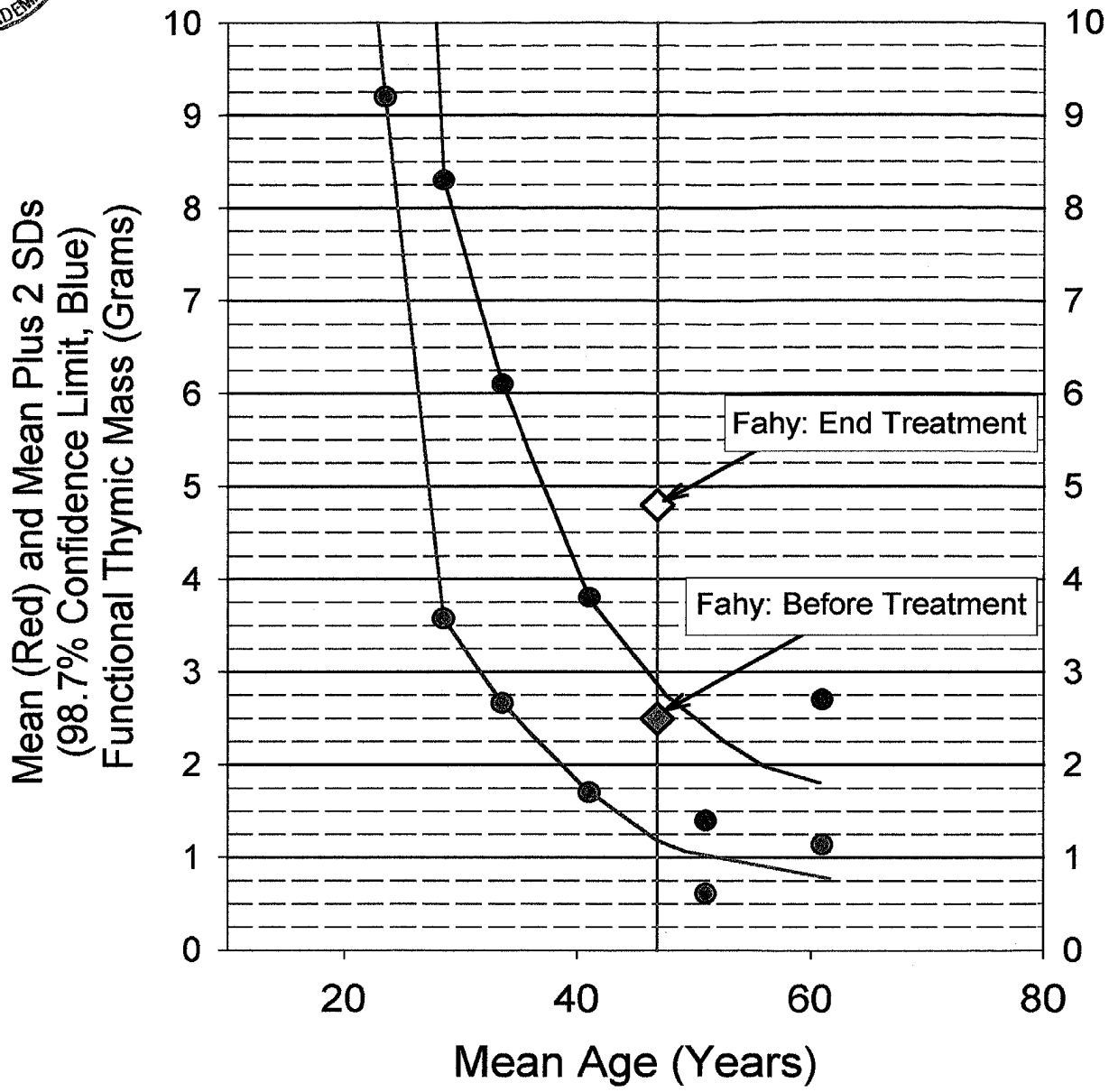

Figure 2: Fahy's functional thymic mass relative to normal values at the age of 46.8 years. Before treatment, the Fahy functional thymic mass fell within the 97.8% confidence limit for normals. After treatment, the Fahy functional thymic mass was well in excess of the 97.8% confidence limit. Thus, the chance that the treatment described in the instant patent application did not regenerate Fahy's thymus is vanishingly small.

glucose levels. As expected, both DHEA and DHEA-S levels rose with treatment, but this did not result in a rise in serum testosterone levels.

Table 2: Response of Serum Analytes to hGH/DHEA Administration

Metabolite (units)	Baseline	Day 8	Day 36
IGF-1 (ng/ml)	235	421	458
Triglycerides (mg/dl)	45	80	68
Total cholesterol (mg/dl)	170	164	138
HDL-cholesterol (mg/dl)	43	50	45
LDL-cholesterol (mg/dl)	118	98	79
VLDL-cholesterol (mg/dl)	9	16	13
DHEA (ng/dl)	308	---	730
DHEA-S (μg/dl)	235	---	638
Testosterone (ng/dl)*	637 [44.6]	430 [20]	391 [26]
Glucose (mg/dl)	87	80	88
T3 (% uptake)	31	33	30
T4 (μg/dl)	7.3	7.6	7.6

*Number in brackets equals 100%x(V-B)/(T-B) where V is the experimental value, B is the bottom of the normal range, and T is the top of the normal range. This figure is given because the normal range for the assay was changed during the study.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further, these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

8/13/2003
Date

Gregory M. Fahy